

Applicants : Jan Geliebter, et al.
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Amendments to the Specification:

Please enter the Sequence Listing attached hereto as **Exhibit 1** (2 pages), and also enclosed herein in computer readable form, as the Sequence Listing for the subject application.

Please amend the paragraph on page 50, line 23, through page 51, line 8, to read as follows:

RT-PCR. To further confirm and detect expression of the recombinant K_{Ca}, two distinct PCR strategies were used: (1) with primers specific to the plasmid sequences (T7 and SP6 promoters); and (2) with primers specific for the 5'-untranslated region (see below). With respect to the latter strategy, oligonucleotide primers for PCR amplification of the 5'-untranslated region (approximately 0.14 kb) were 5'-GCCGCCACCATTTGCCAT-3' (SEQ ID NO:1) (a 3' primer, coding for the first six amino acids of K_{Ca}) and 5'-CCCTATAGTGAGTCGTATTA-3' (SEQ ID NO:2) (a 5' primer, specific to the T7 promoter). With respect to the former strategy, oligonucleotide primers for PCR amplification of the full K_{Ca} insert (approximately 4.2 kb) were the T7 promoter region (see above) and the SP6 promoter region (5'-CTAGCATTTAGGTGACACTATAG-3') (SEQ ID NO:3). The primers for an endogenous K_{Ca} region (bases 909-1074; 5'-GCTCTCCATATTTATCAGCAC-3' (SEQ ID NO:4) and 5'-AACATCCCCATAACCAAC-3' (SEQ ID NO:5)) were used as controls.

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Please amend the paragraph on page 51, line 25, through page 52, line 14, to read as follows:

Total RNA was purified from corporal smooth muscle tissue by TRIzol reagent (Gibco) with glycogen as the carrier. The purified total RNA was subject to RT-PCR, according to the procedure described above. The primer set used for this RT-PCR was 5'-ggctcaagcttctgaggctggtattaagaagtg-3' (SEQ ID NO:6) (forward) and 5'-g'gcacatctagatcctaaattgggttgggagg-3' (SEQ ID NO:7) (reverse). After RT-PCR, the cDNA fragment was TA cloned into pCRII, and then digested with Hind III and XbaI. The pVAX1 (Invitrogen), a 3.0-kb plasmid vector, was used as the expression vector. The pVAX1 was constructed by modifying the pcDNA3.1 vector to use kanamycin instead of ampicillin for selection, so as to avoid the potential pitfall of sensitivity to penicillin when injecting in humans. The unnecessary sequences for replication in *E. coli*, or for expression of the recombinant protein, were also removed. In addition, the pVAX1 was digested by Hind III and XbaI, then treated with alkaline phosphatase to reduce the background. The *Kir6.2* cDNA was then ligated into the pVAX1. The *Kir6.2* cDNA insert was oriented to follow the pCMV β promoter. The cDNA insert was also sequenced to confirm the DNA sequences. The pVAX1/*Kir6.2* construct was subsequently amplified and purified with the Giga prep (Qiagen), as before (Day, *et al.*, *FASEB J.*, 11:A328, 1997).